

# Effects of transgenic Bt cotton on insecticide use and abundance of two generalist predators

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## Abstract

Considerable effort has been expended to determine if crops genetically engineered to produce *Bacillus thuringiensis* (Bt) toxins harm non-target arthropods. However, if Bt crops kill target pests and thereby reduce insecticide use, this could benefit some non-target arthropods. We analyzed data from 21 commercial cotton fields in Arizona to test the effects of Bt cotton on insecticide use and abundance of two non-target arthropods, the generalist predators *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) and *Orius tristicolor* (White) (Heteroptera: Anthocoridae). The number of insecticide sprays was more than double for non-Bt cotton compared with Bt cotton that produced Cry1Ac. The abundance of both predators was negatively associated with the number of insecticide sprays, although significantly so for only one of two sampling periods for each species tested. With the effects of insecticides statistically removed, field type (Bt or non-Bt cotton) did not affect the abundance of either predator. Accordingly, without adjusting for the effects of insecticide sprays, the abundance of *C. carnea* was higher in Bt cotton fields than in non-Bt cotton fields, but significantly so during only one of two sampling periods. The abundance of *O. tristicolor* did not differ between field types, even without adjusting for effects of insecticide sprays. The results indicate that Bt crops can affect insecticide use, which in turn can affect the relative abundance of non-target arthropods in Bt and non-Bt fields. Thus, environmental impact assessment should incorporate analysis of the effects of transgenic crops on management practices, as well as evaluation of the direct effects of such crops.

## Introduction

Crops genetically engineered to produce *Bacillus thuringiensis* (Bt) toxins are planted on millions of hectares worldwide (Lawrence, 2005). Considerable effort has been expended to determine the effects of Bt crops on non-target arthropods (e.g., Zwahlen et al., 2000; Dutton et al., 2002; Al-Deeb & Wilde, 2003; Jasinski et al., 2003; Men et al., 2003; Sisterson et al., 2004, 2007; Sisterson & Tabashnik, 2005; Torres & Ruberson, 2005, 2006). While some negative effects have been detected in the laboratory (e.g., Hilbeck et al., 1998a, 1999; Losey et al., 1999; Ponsard et al., 2002), field studies typically report minor effects (e.g., Al-Deeb

& Wilde, 2003; Jasinski et al., 2003; Men et al., 2003; Sisterson et al., 2004; Dively, 2005; Naranjo, 2005; Torres & Ruberson, 2005; Cattaneo et al., 2006). Effects of Bt crops in the field may be less severe than those observed in the laboratory because exposure to Bt toxins may be lower in the field than in the laboratory (Sears et al., 2001). Thus, extrapolating the results of laboratory studies to the field can be difficult.

In field experiments, plots of Bt and non-Bt cultivars are often managed in the same way so that the only difference between plot types is cultivar (i.e., Bt or non-Bt). However, Bt and non-Bt cultivars of the same crop may be managed differently by growers. In particular, the number of insecticide sprays applied in Arizona was lower for Bt cotton than non-Bt cotton (Carpenter & Gianessi, 2001; Carrière et al., 2001; Cattaneo et al., 2006). Because Bt crops are often less harmful than insecticides to non-target arthropods (Bhatti et al., 2005a,b; Dively, 2005; Naranjo, 2005; Torres

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& Ruberson, 2005; Whitehouse et al., 2005; Cattaneo et al., 2006), reduced insecticide use in Bt crop fields could benefit some non-target species. Consequently, extrapolating the results of controlled field plot studies to growers' fields can be problematic without an understanding of differences in management practices between the two field types.

Here we examined 21 commercial cotton [*Gossypium hirsutum* L. (Malvaceae)] fields in Arizona to compare non-Bt cotton vs. Bt cotton producing Cry1Ac in terms of insecticide use and abundance of two non-target insects, the generalist predators *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) and *Orius tristicolor* (White) (Heteroptera: Anthracoridae). These two natural enemies are common in many cropping systems and are often studied to evaluate effects of transgenic plants on non-target arthropods (Hilbeck et al., 1998a; Zwahlen et al., 2000; Al-Deeb et al., 2001; Ponsard et al., 2002; Romeis et al., 2004; Sisterson et al., 2004; Naranjo, 2005; Rodrigo-Simón et al., 2006). The results show that non-Bt cotton fields received twice as many insecticide sprays as Bt cotton fields and that abundance of both predators was negatively associated with the number of insecticide sprays, but not with field type. These results highlight the importance of considering management differences between Bt and non-Bt crops when evaluating effects on non-target species.

## Materials and methods

### Field sampling

We sampled 21 grower-managed commercial cotton fields in Maricopa Co., AZ, USA, during summer of 2004: 13 fields of transgenic cotton producing Bt toxin Cry1Ac and eight fields of non-Bt cotton. Field types (Bt or non-Bt cotton) were identified based on growers' reports to the Arizona Cotton Research Council. Previous tests confirmed that growers accurately report field types (Carrière et al., 2005). Fields ranged from 1.4 to 35.8 ha (mean = 15.8 ha). Each field was sampled twice, once between 28 July and 24 August and a second time between 11 August and 2 September. The date and the time of day of each sample were recorded. Sampling dates for each sampling period varied widely because we had to accommodate the growers' irrigation and spray schedules. Mean collection dates for each sample did not differ between Bt and non-Bt fields (mean Julian date  $\pm$  SD; first sampling period: Bt  $215.4 \pm 7.4$ , non-Bt  $215.5 \pm 5.3$ ;  $t = 0.04$ , d.f. = 19,  $P = 0.97$ ; second sampling period: Bt  $230.5 \pm 2.1$ , non-Bt  $233.3 \pm 8.8$ ;  $t = 0.79$ , d.f. = 19,  $P = 0.44$ ). Fields were sampled using a sweep net (40 cm in diameter) with 200 sweeps per sample. Samples were placed in 3.8-l sealable bags, taken to the laboratory in a cooler and stored

in a freezer. The number of *C. carnea* and *O. tristicolor* adults were counted using a stereomicroscope. The name *C. carnea* is used broadly following Tauber et al. (2000).

At the end of the growing season, each grower was asked to report the dates and types of insecticides applied during that growing season.

### Statistical analysis

We used multiple regression (SAS, 2001) to evaluate the effects of number of insecticide sprays before a sampling period ( $\log N + 1$  transformed), field type (i.e., Bt or non-Bt), the interaction between these two factors, sampling date (day sample was collected), and sampling time (time of day sample was collected) on arthropod abundance. *Chrysoperla carnea* usually have an adult-dominated age structure in cotton (Rosenheim, 2001). Thus, the number of *C. carnea* adults in a field ( $\log N + 1$  transformed) was the response variable. Analyses for *O. tristicolor* also used the number of adults ( $\log N + 1$  transformed) as the response variable. Four models were fit, corresponding to each sampling period for *C. carnea* and *O. tristicolor*. The interaction term was never significant in such models ( $P > 0.09$ ). Similarly, neither sampling time for *C. carnea* ( $P > 0.78$ ) nor sampling date for *O. tristicolor* ( $P > 0.33$ ) were significantly associated with abundance. Thus, the interaction term and sampling time were not included in inferential models for *C. carnea*; the interaction term and sampling date were excluded in inferential models for *O. tristicolor*.

To test the association between abundance and number of insecticide sprays, the effects of sampling date (*C. carnea*) or time (*O. tristicolor*) on abundance was removed statistically. To do this, residuals from a simple linear regression of abundance on sampling date were obtained (*C. carnea* in first sampling period), partial residuals from a multiple regression of abundance on sampling date, and number of insecticide sprays were calculated (*C. carnea* in second sampling period), partial residuals from a multiple regression of abundance on sampling time, and number of insecticide sprays were calculated (*O. tristicolor* in first sampling period), or residuals from a simple linear regression of abundance on sampling time were obtained (*O. tristicolor* in second sampling period). Partial residuals were used when more than one factor in the regression model was significant because they provide a better estimation of a statistically adjusted response variable (here abundance; Ramsey & Shafer, 2002).

To assess differences in arthropod abundance between Bt and non-Bt fields for each sampling period, we removed statistically the effect of sampling date for *C. carnea* and of sampling time for *O. tristicolor*. This was done by fitting linear regression models relating arthropod abundance to

**Table 1** Average number of insecticide sprays applied in Bt cotton fields, non-Bt cotton fields, and across both field types separated by insecticide class

Insecticide class	Sprays per field		
	Bt cotton	Non-Bt cotton	All cotton
Organophosphate	0.62	0.63	0.62
Pyrethroid	0.08	0.38	0.19
Carbamate	0.23	0.13	0.19
Neonicotinoid	0.00	0.13	0.05
Pyrethroid + Organophosphate	0.00	0.75	0.29
Pyrethroid + Carbamate	0.00	0.38	0.14
Pyrethroid + Oxadiazine	0.00	0.13	0.05
Organophosphate + Neonicotinoid	0.15	0.13	0.14
Total	1.08	2.66	1.67

sampling date for *C. carnea* and to sampling time for *O. tristicolor* and obtaining residuals. Residuals from such models, respectively, represent abundance of *C. carnea* and *O. tristicolor* from which the effect of sampling date or time has been removed. Two-sample t-tests were used to compare the average value of the residuals between field types (i.e., Bt cotton vs. non-Bt cotton).

## Results

### Insecticide use

Compared with Bt cotton fields producing Cry1Ac, non-Bt cotton fields were sprayed 7.1 and 2.4 times more frequently before the first and second sampling periods, respectively [first sampling period (mean  $\pm$  SE): Bt  $0.23 \pm 0.29$ , non-Bt  $1.63 \pm 0.36$ ;  $t = 3.01$ , d.f. = 19,  $P = 0.007$ ; second sampling period (mean  $\pm$  SE): Bt  $1.08 \pm 0.32$ , non-Bt  $2.63 \pm 0.40$ ;  $t = 3.02$ , d.f. = 19,  $P = 0.007$ ]. Sprays that combined insecticides from different classes were more common in non-Bt fields (52%) than in Bt fields (14%) (Table 1;  $\chi^2 = 5.22$ , d.f. = 1,  $P < 0.05$ ).

Bt cotton producing Cry1Ac controls only some lepidopteran pests. Thus, treatments in Bt fields were required to control non-lepidopteran pests such as cotton aphid [*Aphis gossypii* Glover (Homoptera: Aphididae)], western tarnished plant bug [*Lygus hesperus* (Knight) (Heteroptera: Miridae)], and sweet potato whitefly [*Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae)]. In contrast, non-Bt fields required treatments for non-lepidopteran pests and lepidopteran pests. In Arizona, Bt cotton specifically targets the pink bollworm [*Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)], but also suppresses cotton leaf perforator [*Bucculatrix thurberiella* Busck (Lepidoptera: Lyonetiidae)], beet armyworm

[*Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae)], and saltmarsh caterpillar [*Estigmene acrea* (Drury) (Lepidoptera: Arctiidae)] (Wilson et al., 1992). Thus, the reduction in insecticide use in Bt cotton fields can be inferred to be a result of reduced treatments for pink bollworm and other lepidopteran pests that are suppressed on Bt cotton.

### Predator abundance

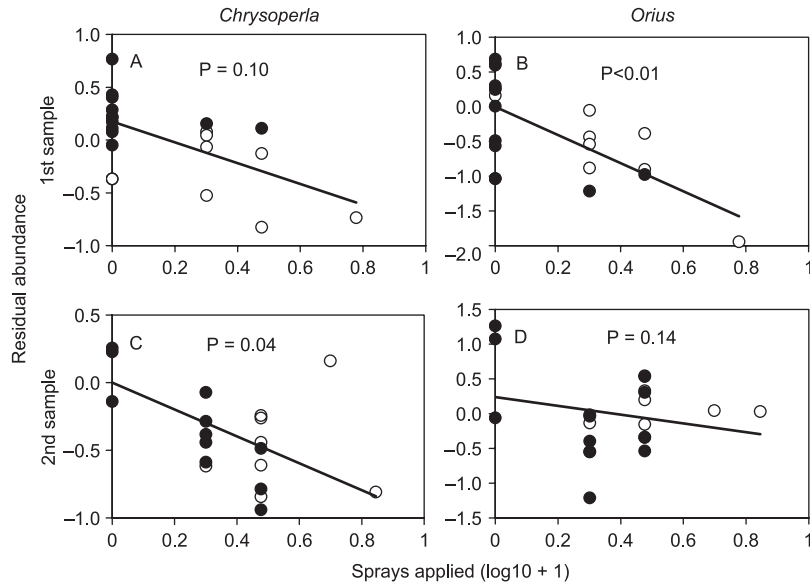
Independent of the effects of field type and number of insecticide sprays, sampling date affected abundance of *C. carnea* in the first (slope = 0.042,  $t = 3.14$ , d.f. = 1,17,  $P = 0.006$ ) and second (slope = 0.029,  $t = 2.26$ , d.f. = 1,17,  $P = 0.037$ ) sampling period, while sampling time affected abundance of *O. tristicolor* in the first (slope = 0.0016,  $t = 2.19$ , d.f. = 1,17,  $P = 0.043$ ) and second (slope = -0.0020,  $t = -2.57$ , d.f. = 1,17,  $P = 0.019$ ) sampling period. Thus, these effects were removed statistically in all analyses.

With the effects of insecticide removed statistically, field type (Bt vs. non-Bt) did not affect the abundance of *C. carnea* or *O. tristicolor* in the first or second sampling periods ( $P > 0.23$ ). In contrast to field type, the number of insecticide sprays was consistently negatively associated with the abundance of *C. carnea* on the first (slope = -0.867,  $t = -1.74$ , d.f. = 1,17,  $P = 0.099$ ) and second (slope = -1.229,  $t = -2.19$ , d.f. = 1,17,  $P = 0.043$ ) sampling period, although the relationship was significant only in the second period (Figure 1A,C). Similarly, the number of insecticide sprays was negatively associated with abundance of *O. tristicolor* in the first (slope = -2.532,  $t = -3.40$ , d.f. = 1,17,  $P = 0.003$ ) and second (slope = -1.173, d.f. = 1,17,  $t = -1.57$ , d.f. = 1,17,  $P = 0.135$ ) sampling period, although the relationship was only significant in the first period (Figure 1B,D).

Without removing effects of insecticide and after correcting for the effect of sampling date, the abundance of *C. carnea* was greater in Bt fields compared to non-Bt fields in both sampling periods (Figure 2A,C), although the effect was only significant for the first sampling period (first sampling period:  $t = 3.79$ , d.f. = 19,  $P = 0.001$ ; second sampling period:  $t = 0.54$ , d.f. = 19,  $P = 0.59$ ). Without removing effects of insecticide and after correcting for the effect of sampling time, the abundance of *O. tristicolor* did not differ significantly between Bt and non-Bt fields in the first ( $t = 1.12$ , d.f. = 19,  $P = 0.28$ ) or second ( $t = -0.40$ , d.f. = 19,  $P = 0.69$ ) sampling period (Figure 2B,D).

## Discussion

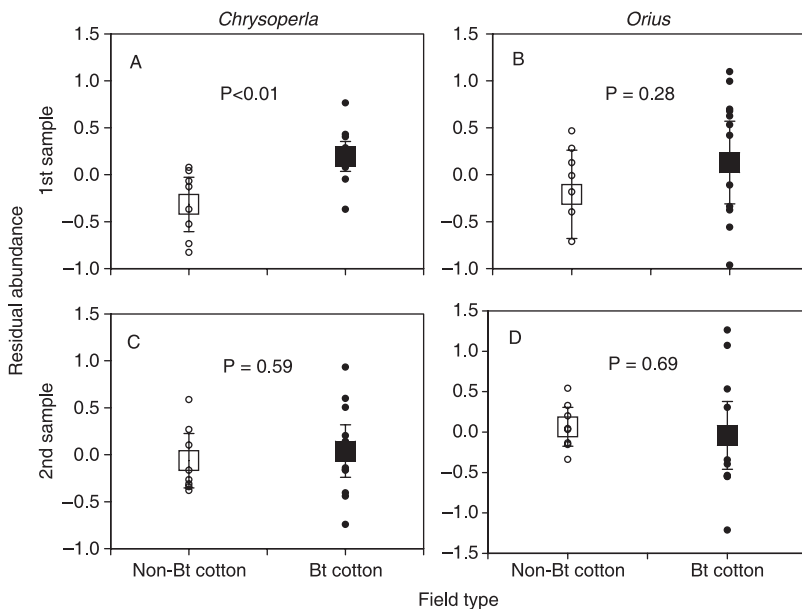
In the 21 Arizona cotton fields studied, insecticide use was lower in fields of Bt cotton producing Cry1Ac than in fields of non-Bt cotton. We also found that abundance of the generalist predators *C. carnea* and *O. tristicolor* was



**Figure 1** Effects of number of insecticide sprays on abundance of adult *Chrysoperla carnea* and *Orius tristicolor* per 200 sweeps in the first and second sampling periods. Effects of sampling date (*C. carnea*) or time (*O. tristicolor*) on abundance were removed statistically to better illustrate the association between abundance and number of insecticide sprays (see Materials and methods). Closed circles represent Bt fields; open circles non-Bt fields. (A) Association between residual abundance of *C. carnea* and number of insecticide sprays in first sampling period, (B) association between partial residual abundance of *O. tristicolor* and number of insecticide sprays in first sampling period, (C) association between partial residual abundance of *C. carnea* and number of insecticide sprays in second sampling period, and (D) association between residual abundance of *O. tristicolor* and number of insecticide sprays in second sampling period.

negatively associated with the number of insecticide sprays during some sampling periods (Figure 1). With the effects of insecticides removed statistically, the type of cotton (Bt vs. non-Bt cotton) did not affect the abundance of either

predator. Without adjusting for effects of insecticide sprays, mean abundance of these two predators was numerically higher in Bt cotton fields in three out of four comparisons between Bt cotton fields and non-Bt cotton fields (Figure 2),



**Figure 2** Average abundance of adult *Chrysoperla carnea* and *Orius tristicolor* per 200 sweeps in Bt and non-Bt cotton fields during the first and second sampling periods. Effects of sampling date (*C. carnea*) or time (*O. tristicolor*) on abundance were removed statistically (see Materials and methods). Circles represent individual observations, squares represent means, and bars 95% confidence intervals.

although this difference was significant only for the first sampling period for *C. carnea* (Figure 2A). Thus, the results suggest that reduced insecticide use in Bt cotton tended to increase abundance of *C. carnea* and *O. tristicolor* in Bt cotton relative to non-Bt cotton.

Because broad spectrum insecticides are frequently used in Arizona cotton (Table 1), other non-target species might also benefit from fewer sprays in Bt cotton fields. However, the impact of Bt cotton could vary among non-target species, depending on their susceptibility to Bt cotton or Bt-intoxicated prey, susceptibility to insecticides, and life-history traits (Sisterson et al., 2007). Effects of Bt cotton on non-target species may also vary depending on which toxins are produced by Bt cotton (i.e., Cry1Ac only, Cry1Ac and Cry2Ab, or Cry1Ac and Cry1F).

*Chrysoperla* species have been the focus of many non-target studies. Some studies suggest that *Chrysoperla* larvae are likely to experience increased mortality when feeding on Bt-intoxicated prey (Hilbeck et al., 1998a,b, 1999) and some do not (Dutton et al., 2002; Romeis et al., 2004; Rodrigo-Simón et al., 2006). Field studies support the latter result, reporting no differences in abundance of *Chrysoperla* species in field plots of unsprayed Bt and non-Bt corn or cotton (corn: Pilcher et al., 1997; Wold et al., 2001; Bourguet et al., 2002; Dively & Rose, 2003; Jasinski et al., 2003; Candolfi et al., 2004; cotton: Flint et al., 1995; Naranjo & Ellsworth, 2003; Hagerty et al., 2005; Naranjo, 2005). Although most studies report no effect, Dively (2005) did report lower abundance of *Chrysoperla* larvae and eggs in plots of Vip3A + Cry1Ab corn relative to conventional corn. He hypothesized that *Chrysoperla* species abundance was lower in Vip3A + Cry1Ab corn fields due to a reduction in prey and host-plant attractancy rather than from direct or indirect mortality caused by the Vip3A + Cry1Ab toxins.

Laboratory studies using Cry1Ab suggest that *Orius* species will not be impacted by Bt toxin (Zwahlen et al., 2000; Al-Deeb et al., 2001). However, a study using Cry1Ac found a negative effect on longevity (Ponsard et al., 2002). Nonetheless, field studies comparing *Orius* species abundance in unsprayed Bt and non-Bt plots report no differences in corn or cotton (corn: Pilcher et al., 1997; Wold et al., 2001; Bourguet et al., 2002; Al-Deeb & Wilde, 2003; Dively & Rose, 2003; Jasinski et al., 2003; Candolfi et al., 2004; Dively, 2005; cotton: Flint et al., 1995; Naranjo & Ellsworth, 2003; Naranjo, 2005).

One of the difficulties confronted by researchers is having to extrapolate the results of laboratory studies to the field. For example, initial laboratory studies suggested that Bt corn could impact monarch butterfly populations (Losey et al., 1999). Subsequent field research indicated that the impact was negligible for most Bt corn varieties

(Sears et al., 2001). Research on lacewings seems to have followed a similar trend. Initial laboratory tests suggested that mortality of lacewing larvae could be higher in Bt corn fields than in non-Bt corn fields (Hilbeck et al., 1998a,b, 1999). However, later laboratory and field studies indicated that the impacts are small (Pilcher et al., 1997; Wold et al., 2001; Bourguet et al., 2002; Dively & Rose, 2003; Jasinski et al., 2003; Candolfi et al., 2004). With regards to Bt cotton, recent laboratory studies found no effect of Cry1Ac on the fitness of *C. carnea* (Rodrigo-Simón et al., 2006). Further, our results suggest that reduced insecticide use in Bt cotton fields could benefit *C. carnea* compared to traditionally managed conventional cotton (Figure 2A). This raises the important question as to what the appropriate controls are for non-target studies.

In another recent study conducted in Arizona, several agronomic factors (plant density, seeding rate, sand content of soil, and insecticide sprays) and ecological factors (plant diversity and biomass in habitats surrounding cotton fields, precipitations) significantly affected the abundance and species richness of ground-dwelling ants and beetles in transgenic and non-transgenic cotton fields (Cattaneo et al., 2006). As in the present study, Bt cotton fields were treated with significantly fewer insecticide sprays than non-Bt cotton fields, while insecticide sprays had significant negative effects on the biodiversity of ants and beetles but crop type did not. In contrast to the present study (Figure 2A), cultivation of transgenic cotton had no significant positive impacts on arthropod abundance (or species richness), showing that assessment of non-target effects of transgenic plants should be done on a case-by-case basis. Nevertheless, both studies illustrate that methods for assessment of impacts of transgenic crops on non-target species should focus on fitness effects of the crops as well as on changes in agricultural practices associated with use of such crops.

While reduced insecticide use in Bt cotton fields has occurred in Arizona, other systems such as Bt corn or Bt cotton in other parts of the USA may not have experienced the same reduction in insecticide use (Carpenter & Gianessi, 2001). Thus, reduced insecticide use in Bt fields may not be observed in all Bt cropping systems. Consequently, our results do not apply to all Bt cropping systems. Nonetheless, our results indicate that differences in management practices in Bt and non-Bt crops should be taken into consideration to fully assess the potential impacts of Bt crops on non-target species.

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